Express Mail No. EV529815955US 10/588570 IAP11 Rec'd PCT/PTO 03 AUG 2006

Sequence Listing

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<120> Mutated DNA polymerases with increased mispairing discrimination
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<150> DE 102004005885.7
<151> 2004-02-05
<160> 29
<170> PatentIn Ver. 2.1
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<211> 2787
<212> DNA
<213> artificial sequence
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cgcccgccaa tgccggacga tctgcgtgca caaatcgaac ccttgcacgc gatggttaaa 300
gcgatgggac tgccgctgct ggcggtttct ggcgtagaag cggacgacgt tatcggtact 360
ctggcgcgcg aagccgaaaa agccgggcgt ccggtgctga tcagcactgg cgataaagat 420
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TNR. 2310 S. 39/61

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<212> PRT

<213> artificial sequence

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<223> Description of artificial sequence: E. coli Klenow fragment of DNA polymerase 1

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Gly Glu Pro Thr Gly Ala Met Tyr Gly Val Leu Asn Met Leu Arg Ser 35 40 45

Leu Ile Met Gln Tyr Lys Pro Thr His Ala Ala Val Val Phe Asp Ala
50 55 60

Lys Gly Lys Thr Phe Arg Asp Glu Leu Phe Glu His Tyr Lys Ser His 65 70 75 80

Arg Pro Pro Met Pro Asp Asp Leu Arg Ala Gln Ile Glu Pro Leu His 85 90

Ala Met Val Lys Ala Met Gly Leu Pro Leu Leu Ala Val Ser Gly Val 100 105 110

Glu Ala Asp Asp Val Ile Gly Thr Leu Ala Arg Glu Ala Glu Lys Ala 115 120 125

Gly Arg Pro Val Leu Ile Ser Thr Gly Asp Lys Asp Met Ala Gln Leu 130 135 140

Val Thr Pro Asn Ile Thr Leu Ile Asn Thr Met Thr Asn Thr Ile Leu 145 150 155 160

Gly Pro Glu Glu Val Val Asn Lys Tyr Gly Val Pro Pro Glu Leu Ile 165 Ile Asp Phe Leu Ala Leu Met Gly Asp Ser Ser Asp Asn Ile Pro Gly Val Pro Gly Val Gly Glu Lys Thr Ala Gln Ala Leu Leu Gln Gly Leu 200 Gly Gly Leu Asp Thr Leu Tyr Ala Glu Pro Glu Lys Ile Ala Gly Leu Ser Phe Arg Gly Ala Lys Thr Met Ala Ala Lys Leu Glu Gln Asn Lys Glu Val Ala Tyr Leu Ser Tyr Gln Leu Ala Thr Ile Lys Thr Asp Val Glu Leu Glu Leu Thr Cys Glu Gln Leu Glu Val Gln Gln Pro Ala Ala Glu Glu Leu Leu Gly Leu Phe Lys Lys Tyr Glu Phe Lys Arg Trp Thr 280 Ala Asp Val Glu Ala Gly Lys Trp Leu Gln Ala Lys Gly Ala Lys Pro Ala Ala Lys Pro Gln Glu Thr Ser Val Ala Asp Glu Ala Pro Glu Val 310 315 Thr Ala Thr Val Ile Ser Tyr Asp Asn Tyr Val Thr Ile Leu Asp Glu 325 Glu Thr Leu Lys Ala Trp Ile Ala Lys Leu Glu Lys Ala Pro Val Phe 345 Ala Phe Asp Thr Glu Thr Asp Ser Leu Asp Asn Ile Ser Ala Asn Leu Val Gly Leu Ser Phe Ala Ile Glu Pro Gly Val Ala Ala Tyr Ile Pro Val Ala His Asp Tyr Leu Asp Ala Pro Asp Gln Ile Ser Arg Glu Arg Ala Leu Glu Leu Leu Lys Pro Leu Leu Glu Asp Glu Lys Ala Leu Lys Val Gly Gln Asn Leu Lys Tyr Asp Arg Gly Ile Leu Ala Asn Tyr Gly Ile Glu Leu Arg Gly Ile Ala Phe Asp Thr Met Leu Glu Ser Tyr Ile Leu Asn Ser Val Ala Gly Arg His Asp Met Asp Ser Leu Ala Glu Arg Trp Leu Lys His Lys Thr Ile Thr Phe Glu Glu Ile Ala Gly Lys Gly 470

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Lys Asn Gln Leu Thr Phe Asn Gln Ile Ala Leu Glu Glu Ala Gly Arg Tyr Ala Ala Glu Asp Ala Asp Val Thr Leu Gln Leu His Leu Lys Met 505 Trp Pro Asp Leu Gln Lys His Lys Gly Pro Leu Asn Val Phe Glu Asn 520 Ile Glu Met Pro Leu Val Pro Val Leu Ser Arg Ile Glu Arg Asn Gly Val Lys Ile Asp Pro Lys Val Leu His Asn His Ser Glu Glu Leu Thr 555 Leu Arg Leu Ala Glu Leu Glu Lys Lys Ala His Glu Ile Ala Gly Glu 565 Glu Phe Asn Leu Ser Ser Thr Lys Gln Leu Gln Thr Ile Leu Phe Glu Lys Gln Gly Ile Lys Pro Leu Lys Lys Thr Pro Gly Gly Ala Pro Ser Thr Ser Glu Glu Val Leu Glu Glu Leu Ala Leu Asp Tyr Pro Leu Pro Lys Val Ile Leu Glu Tyr Arg Gly Leu Ala Lys Leu Lys Ser Thr Tyr 630 Thr Asp Lys Leu Pro Leu Met Ile Asn Pro Lys Thr Gly Arg Val His 650 Thr Ser Tyr His Gln Ala Val Thr Ala Thr Gly Arg Leu Ser Ser Thr Asp Pro Asn Leu Gln Asn Ile Pro Val Arg Asn Glu Glu Gly Arg Arg 680 Ile Arg Gln Ala Phe Ile Ala Pro Glu Asp Tyr Val Ile Val Ser Ala Asp Tyr Ser Gln Ile Glu Leu Arg Ile Met Ala His Leu Ser Arg Asp Lys Gly Leu Leu Thr Ala Phe Ala Glu Gly Lys Asp Ile His Arg Ala Thr Ala Ala Glu Val Phe Gly Leu Pro Leu Glu Thr Val Thr Ser Glu 745 Gln Arg Arg Ser Ala Lys Ala Ile Asn Phe Gly Leu Ile Tyr Gly Met 760 Ser Ala Phe Gly Leu Ala Arg Gln Leu Asn Ile Pro Arg Lys Glu Ala

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Gln Lys Tyr Met Asp Leu Tyr Phe Glu Arg Tyr Pro Gly Val Leu Glu
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            820
                                825
Gly Ala Arg Arg Ala Ala Ala Glu Arg Ala Ala Ile Asn Ala Pro Met
                            840
Gln Gly Thr Ala Ala Asp Ile Ile Lys Arg Ala Met Ile Ala Val Asp
Ala Trp Leu Gln Ala Glu Gln Pro Arg Val Arg Met Ile Met Gln Val
                    670
                                        875
His Asp Glu Leu Val Phe Glu Val His Lys Asp Asp Val Asp Ala Val
                                    890
Ala Lys Gln Ile His Gln Leu Met Glu Asn Cys Thr Arg Leu Asp Val
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<211> 2499
<212> DNA
<213> artificial sequence
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<223> Description of artificial sequence: Wild type Taq polymerase
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gtgcaggcgg tctacggctt cgccaagagc ctcctcaagg ccctcaagga ggacggggac 180
gcggtgatcg tggtctttga cgccaaggcc ccctccttcc gccacgaggc ctacgggggg 240
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gagetggtgg acetectggg getggegee etegaggtee egggetaega ggeggaegae 360
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gccctcaggg acctgaagga ggcgcggggg cttctcgcca aagacctgag cgttctggcc 1080
ctgagggaag goottggcot cocgcocggc gacgacccca tgctcctcgc ctacctcctg 1140
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<211> 832
<212> PRT
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<223> Description of artificial sequence: Wild type Taq polymerase

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Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala

Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile Val

Val Phe Asp Ala Lys Ala Pro Ser Phe Arg His Glu Ala Tyr Gly Gly

Tyr Lys Ala Gly Arg Ala Pro Thr Pro Glu Asp Phe Pro Arg Gln Leu

Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Leu Ala Arg Leu Glu

Val Pro Gly Tyr Glu Ala Asp Asp Val Leu Ala Ser Leu Ala Lys Lys 120

<213> Artificial sequence

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 Glu Gly
 Tyr
 Glu 135
 Val Arg
 Ile Leu Thr 140
 Ala Asp Lys
 Asp Lys
 Asp 116

 Leu Tyr
 Gln Leu Leu Leu 150
 Leu Ser Asp 150
 Arg 11e His Not 155
 Val Leu His Pro Glu Gly 160
 Gly 160

 Tyr
 Leu Ile Thr 165
 Ala Trp Leu Trp 165
 Ala Trp Arg Arg Arg Leu 170
 Tyr Gly Leu Arg 175
 Pro Rap Arg Arg Arg Arg Arg Arg Arg Arg Arg 180
 Thr Gly Asp Glu Ser Asp Asp Arg Leu Leu 195
 Asp 190
 Asp Arg Leu Leu Leu 195
 Asp Arg Leu Leu 195
 Arg Leu Leu 205
 Asp Arg Leu Leu 205
 Leu Lys Asp Leu Asp Arg Leu 240
 Leu Lys 235
 Asp Leu Pro Leu Glu Val 255
 Val 245
 Arg Thr 250
 Asp Leu Pro Leu Glu Val 255
 Val 255
 Asp Leu Pro Leu Glu Val 255
 Val 255
 Val 255
 Asp Leu Pro Leu Glu Val 255
 Val

Asp Phe Ala Lys Arg Arg Glu Pro Asp Arg Glu Arg Leu Arg Ala Phe

Leu Glu Arg Leu Glu Phe Gly Ser Leu Leu His Glu Phe Gly Leu Leu 275 280 285

Glu Ser Pro Lys Ala Leu Glu Glu Ala Pro Trp Pro Pro Pro Glu Gly 290 295 300

Ala Phe Val Gly Phe Val Leu Ser Arg Lys Glu Pro Met Trp Ala Asp 305 310 315 320

Leu Leu Ala Leu Ala Ala Ala Arg Gly Gly Arg Val His Arg Ala Pro 325 330 335

Glu Pro Tyr Lys Ala Leu Arg Asp Leu Lys Glu Ala Arg Gly Leu Leu 340 345 350

Ala Lys Asp Leu Ser Val Leu Ala Leu Arg Glu Gly Leu Gly Leu Pro 355 360 365

Pro Gly Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro Ser Asn 370 380

Thr Thr Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp Thr Glu 385 390 395 400

Glu Ala Gly Glu Arg Ala Ala Leu Ser Glu Arg Leu Phe Ala Asn Leu
405 410 415

Trp Gly Arg Leu Glu Gly Glu Glu Arg Leu Leu Trp Leu Tyr Arg Glu
420 425 430

Val Glu Arg Pro Leu Ser Ala Val Leu Ala His Met Glu Ala Thr Gly 435 440 445

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Val Arg Leu Asp Val Ala Tyr Leu Arg Ala Leu Ser Leu Glu Val Ala Glu Glu Ile Ala Arg Leu Glu Ala Glu Val Phe Arg Leu Ala Gly His 470 475 Pro Phe Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg Val Leu Phe Asp Glu Leu Gly Leu Pro Ala Ile Gly Lys Thr Glu Lys Thr Gly Lys Arg Ser Thr Ser Ala Ala Val Leu Glu Ala Leu Arg Glu Ala His Pro Ile 520 Val Glu Lys Ile Leu Gln Tyr Arg Glu Leu Thr Lys Leu Lys Ser Thr Tyr Ile Asp Pro Leu Pro Asp Leu Ile His Pro Arg Thr Gly Arg Leu His Thr Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu Ser Ser 570 Ser Asp Pro Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu Gly Gln 585 Arg Ile Arg Arg Ala Phe Ile Ala Glu Glu Gly Trp Leu Leu Val Ala Leu Asp Tyr Ser Glm Ile Glu Leu Arg Val Leu Ala His Leu Ser Gly Asp Glu Asn Leu Ile Arg Val Phe Gln Glu Gly Arg Asp Ile His Thr Glu Thr Ala Ser Trp Met Phe Gly Val Pro Arg Glu Ala Val Asp Pro Leu Met Arg Arg Ala Ala Lys Thr Ile Asn Phe Gly Val Leu Tyr Gly 665 Met Ser Ala His Arg Leu Ser Gln Glu Leu Ala Ile Pro Tyr Glu Glu 680 Ala Gln Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys Val Arg 695 Ala Trp Ile Glu Lys Thr Leu Glu Glu Gly Arg Arg Gly Tyr Val Glu Thr Leu Phe Gly Arg Arg Arg Tyr Val Pro Asp Leu Glu Ala Arg 730 Val Lys Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn Met Pro

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Val Gln Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys Leu 755 760 765

Phe Pro Arg Leu Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val His
770 780

Asp Glu Leu Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val Ala 785 790 795 800

Arg Leu Ala Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val Pro 805 810 815

Leu Glu Val Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys Glu 820 825 830

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<211> 39

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<223> Description of artificial sequence: Primer

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39

<210> 6

<211> 23

<212> DNA

<213> Artificial sequence

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<223> Description of artificial sequence:
 Downstream primer

<400> 6

gctaattaag cttggctgca ggc

23

<210> 7

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Description of artificial sequence:
 Antisense primer

<400> 7

tacatggacc tttacttcga acgc

24

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<210> 8
<211> 20
<212> DNA
<213> Artificial sequence
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<223> Description of artificial sequence: Primer
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<400> 8
acaaaatacc tgtattcctt
                                                                    20
<210> 9
<211> 90
<212> DNA
<213> Artificial sequence
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<223> Description of artificial sequence: Template
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tccctggaca ggcaaggaat acaggtattt
<210> 10
<211> 90
<212> DNA
<213> Artificial sequence
<220>
<223> Description of artificial sequence: Template
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<400> 10
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tccctggaca ggcgaggaat acaggtattt
<210> 11
<211> 20
<212> DNA
<213> Artificial sequence
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<223> Description of artificial sequence: Primer for the
      detection of the SNP in human genomic factor V
      Leiden DNA sequence
<400> 11
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                                                                   20
<210> 12
<211> 35
<212> DNA
<213> Artificial sequence
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<223> Description of artificial sequence: Template of human genomic factor V Leiden DNA sequence; n = g, wild type template; n = a, mutant template <400> 12 gatccctgga caggcnagga atacaggtat tttgt 35 <210> 13 <211> 22 <212> DNA <213> Artificial sequence <223> Description of artificial sequence: Primer for the detection of human somatic ERAF T1796A mutation <400> 13 gacccactcc atcgagattt ct 22 <210> 14 <211> 35 <212> DNA <213> Artificial sequence <220> <223> Description of artificial sequence: Wild type template of BRAF gene; w = t, wild type template; w a, mutant template <400> 14 ggtctagcta cagwgaaatc tcgatggagt gggtc 35 <210> 15 <211> 25 <212> DNA <213> Artificial sequence <223> Description of artificial sequence: Primer for the detection of human dihydropyrimidine dehydrogenase (DPyD) mutation G735A <400> 15 gttttagatg ttaaatcaca cttat 25 <210> 16 <211> 38 <212> DNA <213> Artificial sequence <220>

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<223> Description of artificial sequence: Template of human DPyD; r = g, wild type template; r = a, mutant template <400> 16 ctttccagac aacrtaagtg tgatttaaca tctaaaac 38 <210> 17 <211> 20 <212> DNA <213> Artificial sequence <223> Description of artificial sequence: Primer for the detection of human acid ceramidase mutation A107G <400> 17 cgttggtcct gaaggaggat 20 <210> 18 <211> 33 <212> DNA <213> Artificial sequence <220> <223> Description of artificial sequence: Template of human acid ceramidase; r = a, wild type template; r = g, mutant template <400> 18 aaatcaacct rtcctccttc aggaccaacg tac 33 <210> 19 <211> 22 <212> DNA <213> Artificial sequence <220> <223> Description of artificial sequence: Primer probe BrafT <400> 19 gacccactcc atcgagattt ct 22 <210> 20 <211> 22 <212> DNA <213> Artificial sequence <220> <223> Description of artificial sequence: Reverse primer for BRAF <400> 20 22 agaggaaaga tgaagtacta tg

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<210> 21
<211> 239
<212> DNA
<213> Artificial sequence
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<223> Description of artificial sequence: Target template
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acctattttt actgtgaggt cttcatgaag aaatatatct gaggtgtagt aagtaaagga 120
aaacagtaga totcatttto otatoagago aagcattatg aagagtttag gtaagagato 180
taatttetat aattetgtaa tataatatte tittaaaacat agtaetteat ettteetet 239
<210> 22
<211> 25
<212> DNA
<213> Artificial sequence
<220>
<223> Description of artificial sequence: Primer probe
      DpyDT
<400> 22
gttttagatg ttaaatcaca cttat
                                                                   25
<210> 23
<211> 23
<212> DNA
<213> Artificial sequence
<220>
<223> Description of artificial sequence: Reverse
      primer for DpyDT
<400> 23
aaagctcctt tctgaatatt gag
                                                                   23
<210> 24
<211> 300
<212> DNA
<213> Artificial sequence
<223> Description of artificial sequence: Target template
      DpyDX; r = a, DpyDA (wild type); r = t, DpyDT (mutant)
<100> 24
aaaatgtgag aagggacctc ataaaatatg tcatatggaa atgagcagat aataaagatt 60
atagcttttc tttgtcaaaa ggagactcaa tatctttact ctttcatcag gacattgtga 120
caaatgtttc ccccagaatc atccggggaa ccacctctgg ccccatgtat ggccctggac 180
aaagctcctt totgaatatt gagctcatca gtgagaaaac ggctgcatat tggtgtcaaa 240
gtgtcactga actaaagget gactttccag acaacrtaag tgtgatttaa catctaaaac 300
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<210> 25
<211> 7043
<212> DNA
<213> Artificial sequence
<220>
<223> Description of artificial sequence: pTTQ18::Tag
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<213> Artificial sequence
<223> Description of artificial sequence:
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      wild type; r = a, mutant
<400> 28
gtgaatrcaa c
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<210> 29
<211> 11
<212> DNA
<213> Artificial sequence
<220>
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<223> Description of artificial sequence: Template segment (SEQ ID NO: 12); r=t, wild type; r=a, mutant

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